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(RESEARCH ARTICLE)

Valorisation of bamboo stems for the production of fermentable carbohydrates

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Abstract

Fermentable carbohydrates serve as the primary raw material for the majority of bio-based commodity chemicals and biofuels. The demand for this raw material is projected to rise significantly in the coming decades. The anticipated rise in demand for fermentable carbohydrates (FC), also known as fermentable sugars (FS), is indicated by the numerous biochemical pathways being developed for the production of bio-based commodity chemicals through sugar fermentation. The high cellulose and hemicellulose content in bamboo stems makes them a promising source for producing fermentable sugars, which is the aim of this project, to valorise bamboo stems for the production of fermentable sugars. A small amount of the bamboo stem sample (20 mg) was dried (55 °C, 10 mbar, 2 h) using a rotational vacuum concentrator (Christ Rvc 2-33IR), the dried substance was dissolved in 1000 µL of pyridine and placed in an ultrasonic bath for 5 min. In addition, the sample was mixed well by vortex for 20 s. The sample was centrifuged (12,000× g, 10 min, 20 °C) to remove any insoluble materials. Part of the supernatant, 800 µL, was taken for the oximation-silylation step. Pretereatment was by enzymatic hydrolysis and a gas chromatography-mass spectrometry (GC-MS) analysis was carried out, several carbohydrates were identified, including D-Galacturonic acid, D-Glucuronic acid, D-Fructose, L-Rhamnose, D-Glucose, D-Galactose, D-Mannose, L-Arabinose, D-Fucose, D-Xylose, D-Glucosamine hydrochloride, D-Galactosamine hydrochloride, D-Mannosamine hydrochloride, and Meso-Erythritol. The fermentation was carried out for 28 days and measurements were taken every 7 days. The fermentation process demonstrated a progressive increase in sugar concentrations, with D-Glucose and D-Xylose reaching their highest concentrations on Day 28, with peak areas of 23.41 and 31.77 respectively. The results confirm that bamboo stems are a viable and cost-effective lignocellulosic biomass contributing to effective waste management, renewable energy development and climate change mitigation efforts.

Keywords: Valorisation; Bamboo Stems; Fermentable Carbohydrates; Bio-Based Chemicals; Sustainability

1. Introduction

Lignocellulosic resources have the capacity to function as sustainable energy sources and bio-based materials for biorefineries, therefore mitigating the strain imposed by present energy demands [1, 2]. The primary objectives of the biorefinery process are to optimise material value extraction and minimise production expenses [3]. Prior investigations into biorefineries have predominantly concentrated on agricultural waste [4], whereas there is a paucity of studies addressing the utilisation of food processing waste. Historically, bamboo stems were burnt and disposed of in landfills instead of being used efficiently, leading to significant resource wastage. The bamboo stem offers significant economic advantages compared to other lignocellulosic materials used as energy sources [4, 5]. Bamboo stem, as a category of food processing waste, is cost-effective to get. Moreover, direct procurement from manufacturers reduces the elevated expenses associated with recycling. The substantial cellulose and hemicellulose content in bamboo stems significantly increases the economic worth of their biological refining. The partial development and use of hemicellulose will provide added value that aids in offsetting the economically unviable synthesis of ethanol from cellulose [6].

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Production expenses must be taken into account when choosing the pretreatment technique for bamboo stems, and the economic value of cellulose and hemicellulose post-pretreatment should be preserved to the greatest extent feasible [4].

The majority of bamboo species thrive in warm, humid tropical and mild temperate climates [7]. Bamboo has a growth rate of 30 to 100 cm per day throughout the growing season, making it one of the fastest-growing plants on the planet [8, 9]. Bamboo plants can attain a height of 5 to 25 meters within a growth period of 2 to 4 months [10]. The rapid growth rate of bamboo obstructs the development of other plants by restricting their access to nearby nutrients and sunshine while consuming available water. Consequently, bamboo is expected to diminish species diversity as a result of ecological disturbances. Global warming exacerbates this danger by promoting the expansion of bamboo forests into new regions. Lignocellulosic biomass resources, such as bamboo, are being extensively researched globally as substitutes for fossil fuels in industrial fuel generation inside biorefineries [11]. The primary constituents of lignocellulose in bamboo species are cellulose (38–50%), hemicellulose (23–32%), and lignin (15–25%) [12]. The lignin concentration in woody and herbaceous plants exhibits significant variation in chemical composition. Herbaceous plants have roughly 8–15% lignin, whereas woody plants possess about 20–38% lignin [13]. Bamboo, although being a herbaceous species, has a lignin level comparable to that of woody plants [14]. These components are synthesised by photosynthesis using atmospheric carbon dioxide [11]. Among these components, cellulose has lately emerged as a vital resource for biorefineries [15]. In a biorefinery, glucose generated from cellulose hydrolysis is transformed into an array of white compounds during a following fermentation process using diverse microbial species. These compounds may replace those generated by oil refineries [16]. Bioethanol, a well recognised biorefinery product, is derived from lignocellulosic biomass and has been commercialised [17]. Bioethanol may substitute petroleum-based transportation fuels, so potentially mitigating the adverse impacts of climate change by decreasing greenhouse gas emissions, particularly carbon dioxide.

The increasing demand for clean and renewable energy sources has created significant pressure to identify alternative feedstocks for biofuel production, this calls for this study aimed at optimizing the valorization of bamboo stems by efficiently converting them into fermentable carbohydrates.

2. Materials and methods

2.1. Equipment, Materials, and Reagents

- Auto sampler vials, 150 µL vial inserts, and crimp seals
- Vial crimper and decrimper
- 2.5mL airtight syringe or 3mL disposable hypodermic syringe
- 10 micro-liter autosampler syringe
- 30-m x 0.25-mm or 0.32-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5 or equivalent), 1-µm film thickness.
- Ultrasonic bath
- Centrifuge
- Vortex
- Agilent 6890N Gas Chromatograph with Agilent 5975 Mass Selective Detector

2.2. Sample Preparation/Pretreatment

A small amount of the sample (20 mg) was dried (55 °C, 10 mbar, 2 h) using a rotational vacuum concentrator (Christ Rvc 2-33IR). The dried substance was dissolved in 1000 µL of pyridine and placed in an ultrasonic bath for 5 min. In addition, the sample was mixed well by vortex for 20 s. The sample was centrifuged (12,000× *g*, 10 min, 20 °C) to remove any insoluble materials. Part of the supernatant, 800 µL, was taken for the oximation-silylation step.

2.3. Standard Sugar Preparation

Standard sugar solutions were prepared separately by dissolving 10 mg of sugar (glucose, fructose, mannose, or sucrose etc) in 800 µL of pyridine. For GCMS analysis, these solutions were subjected to the oximation-silylation step.

2.3.1. Procedure

Hexose sugars, such as glucose, fructose and mannose, in standard solutions or sugars in the samples were oximated prior to GCMS analysis. The oximation was achieved by adding 50 mg of O-methyl hydroxylamine-hydrochloride to 800 µL of the standard sugar solution or sample solution. The solutions were incubated at 95 °C for 45 min on a heat plate.

Then, 200 µL of the silylation reagent, N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), was added to each oximated sample. The mixture was incubated at 90 °C for 30 min on a heat plate. Then, the samples were centrifuged (8000× *g*, 10 min, 20 °C). The standard sucrose solution was silylated following the same procedure. However, the incubation time was extended to 60 min. Finally, the derivatized samples were transferred to the GCMS device for analysis.

GC–MS analysis was carried out using an Agilent 6890 gas chromatograph with a 5973 MS detector equipped 30-m x 0.25-mm or 0.32-mm ID fused-silica capillary colum chemically bonded with SE-54 (DB-5 or equivalent), 1-µm film thickness. (Agilent). The following temperature ramp was used: injector at 250 °C, oven initially at 200 °C, held for 1 min and heated to 230 °C (1.5 °C min-1, then held for 10 min). The characterization and identification of the sugar, from the sample was completed in the SCAN mode with the m/z range varied from 35 to 450. The flow rate of the nitrogen as carrier gas was 1 mL min-1; The derivatized sample (1 µL) was automatically injected into the GCMS. The composition of the sugars from the sample was determined using an Agilent 6820 gas chromatograph equipped with 30-m x 0.25-mm or 0.32-mm ID fused.

3. Results and discussion

3.1. Carbohydrate Content of Bamboo Stems

The GC-MS analysis reveals the presence of several carbohydrates in the bamboo stems throughout the fermentation process. The compounds identified include:

D-Galacturonic acid, D-Glucuronic acid, D-Fructose, L-Rhamnose, D-Glucose, D-Galactose, D-Mannose, L-Arabinose, D-Fucose, D-Xylose, D-Glucosamine hydrochloride, D-Galactosamine hydrochloride, D-Mannosamine hydrochloride, and Meso-Erythritol.

The results confirm that bamboo stems contain a variety of sugars and sugar acids, which are key fermentable carbohydrates. Tse et al. Stated that enzymatic hydrolysis releases fermentable monosaccharide and disaccharide sugars. Yeast then converts these sugars (e.g., glucose, galactose, and fructose) to ethanol, carbon dioxide, and other by-products in metabolic processes that can occur under both aerobic and anaerobic conditions. In particular, D-Glucose and D-Xylose are among the most abundant, and these sugars are critical for biofuel production, they are a primary substrate for fermentation into bioethanol and other biofuels as stated by Ambaye et al. [18].

3.2. Concentration of Identified Compounds

Table 1 Fermentation Results for Day 0 of Fermentation

Compound Identified	R.T (min)	Peak Area
D-Galacturonic acid	8.874	0.00
D-Glucuronic acid	10.394	0.00
D-Fructose	10.784	0.00
L-Rhamnose	10.962	0.00
D-Glucose	12.291	0.00
D-Galactose	12.774	0.00
D-Mannose	13.290	0.00
L-Arabinose	13.824	0.00
D-Fucose	14.387	0.00
D-Xylose	14.814	0.00
D-Glucosamine hydrochloride	15.720	0.00
D-Galactosamine hydrochloride	17.418	0.00
D-Mannosamine hydrochloride	17.926	0.00
Meso-Erythritol	18.742	0.00

The tables presented above detail the retention times and peak areas of the identified compounds, providing direct measurements of the concentration of each compound at Days 0, 7, 14, 21, and 28 throughout the fermentation period. Retention time (R.T.) is an essential metric in gas chromatography (GC) that indicates the duration required for a compound to traverse the GC column and arrive at the detector. The retention time of each compound is determined by its specific chemical properties, facilitating its identification within a mixture (qualitative analysis). The peak area is directly proportional to the concentration of a compound within the sample; an increase in peak area signifies an increase in the compound's concentration (quantitative) [19]. The combination of retention time and peak area serves as an effective metric for quantifying the compounds generated throughout the fermentation process.

At the initiation of the fermentation process, as illustrated in Table 4.1, there were no detectable fermentable compounds generated, as evidenced by the peak area of 0.00 for all identified compounds. The bamboo stems at this stage had not yet experienced adequate enzymatic hydrolysis or fermentation to yield fermentable sugars, as this was Day 0.

Table 2 Fermentation Results for Day 7 of Fermentation

Compound Identified	R.T (min)	Peak Area
D-Galacturonic acid	8.742	0.01
D-Glucuronic acid	10.296	0.08
D-Fructose	10.474	0.03
L-Rhamnose	10.821	0.04
D-Glucose	12.470	0.11
D-Galactose	12.913	0.04
D-Mannose	13.374	0.01
L-Arabinose	13.862	0.09
D-Fucose	14.279	0.03
D-Xylose	14.842	0.02
D-Glucosamine hydrochloride	15.963	0.04
D-Galactosamine hydrochloride	17.314	0.01
D-Mannosamine hydrochloride	17.785	0.03
Meso-Erythritol	18.692	0.06

On Day 7, small amounts of the fermentable compounds were detected, signifying the initial phases of bamboo decomposition and sugar liberation (Table 4.2). D-Glucose exhibited a peak area of 0.11, indicating a low concentration at this measurement, although it represented the highest peak area observed among the other compounds on Day 7. D-Glucose serves as the main monosaccharide produced during the hydrolysis of cellulose. The retention times were in alignment with the anticipated values for each compound. The observed increase in peak areas indicates a clear presence of the compounds by Day 7. While the enzymatic hydrolysis proved to be effective, its overall effectiveness remains somewhat constrained.

Table 3 Fermentation Results for Day 14 of Fermentation

Compound Identified	R.T. (Min)	Peak Area
D-Galacturonic acid	8.759	1.52
D-Glucuronic acid	10.264	2.11
D-Fructose	10.532	2.63
L-Rhamnose	10.894	1.28

D-Glucose	12.303	7.41
D-Galactose	12.684	1.89
D-Mannose	13.452	2.05
L-Arabinose	13.758	1.20
D-Fucose	14.831	1.11
D-Xylose	14.831	13.45
D-Glucosamine hydrochloride	15.974	0.51
D-Galactosamine hydrochloride	17.693	0.25
D-Mannosamine hydrochloride	17.974	0.19
Meso-Erythritol	18.617	1.48

Notable increases in peak area were recorded on Day 14, indicating a rapid breakdown of the bamboo structure and subsequent sugar release. D-Xylose exhibited the highest concentration, evidenced by a significant rise in its peak area from 0.02 (Table 4.2) on day 7 to 13.45 on day 14 (Table 4.3). D-Glucose exhibited a peak area of 7.41, ranking as the second most abundant compound. Additionally, it demonstrated a significant increase in concentration relative to Day 7 of the fermentation process. The increase in peak areas is associated with elevated sugar concentrations, indicating that enzymatic hydrolysis was achieving greater efficiency during this phase.

Table 4 Fermentation Results for Day 21 of Fermentation

Compound Identified	**R.T. (Min)**	**Peak Area**
D-Galacturonic acid	8.837	4.64
D-Glucuronic acid	10.314	3.17
D-Fructose	10.695	1.84
L-Rhamnose	10.877	1.56
D-Glucose	12.408	17.43
D-Galactose	12.751	1.65
D-Mannose	13.296	1.85
L-Arabinose	13.855	0.94
D-Fucose	14.382	1.75
D-Xylose	14.764	24.45
D-Glucosamine hydrochloride	15.830	5.20
D-Galactosamine hydrochloride	17.362	3.74
D-Mannosamine hydrochloride	17.794	2.53
Meso-Erythritol	18.846	1.85

On Day 21, the concentrations of D-Glucose and D-Xylose, as indicated in Table 4.4, exhibited a significant increase, reaching peak areas of 17.43 and 24.45, respectively. The elevated levels observed on this day of fermentation indicate the ideal release of fermentable sugars. The retention times exhibited stability, while acid and D-Glucuronic acid, also increased in concentration, reflecting the breakdown of pectins in the bamboo stems.

Table 5 Fermentation Results for Day 28 of Fermentation

Compound Identified	R.T. (Min)	Peak Area
D-Galacturonic acid	8.694	6.76
D-Glucuronic acid	10.327	4.81
D-Fructose	10.564	4.63
L-Rhamnose	10.857	3.79
D-Glucose	12.338	23.41
D-Galactose	12.947	2.89
D-Mannose	13.634	3.42
L-Arabinose	13.905	1.84
D-Fucose	14.256	3.28
D-Xylose	14.694	31.77
D-Glucosamine hydrochloride	15.782	6.36
D-Galactosamine hydrochloride	17.339	4.96
D-Mannosamine hydrochloride	17.885	5.79
Meso-Erythritol	18.752	2.65

D-Glucose and D-Xylose were the most abundant fermentable carbohydrates, with peak areas reaching 23.41 and 31.77 by Day 28, respectively. This highlights their dominance in the fermentation process and their critical role as substrates for bioethanol production.

4. Conclusion

This project on the valorisation of bamboo stems for the production of fermentable carbohydrates successfully demonstrated the potential of bamboo as a sustainable biomass for biofuel production. The GC-MS analysis revealed the presence of several important fermentable compounds throughout the fermentation process, including D-Glucose, D-Xylose, and various other sugars and sugar acids, such as D-Galacturonic acid and D-Glucuronic acid.

The results from the fermentation process showed a clear progression in the breakdown of bamboo's lignocellulosic structure leading to a significant release of fermentable sugars. Starting from Day 0, when no detectable sugars were observed, the concentrations of these compounds increased steadily by Day 7, with a marked acceleration in sugar release by Day 14, and peaking at Day 28. D-Glucose and D-Xylose were the most abundant fermentable carbohydrates, with peak areas reaching 23.41 and 31.77 by Day 28, respectively. This highlights their dominance in the fermentation process and their critical role as substrates for bioethanol production.

The fermentation process, combined with enzymatic hydrolysis, proved to be effective in converting the complex carbohydrate structures of bamboo into simple sugars. The retention times remained stable throughout the study, confirming consistent identification of the compounds, while the increasing peak areas demonstrated the successful breakdown and release of sugars over time.

In conclusion, the valorisation of bamboo systems offers a promising pathway for the production of fermentable carbohydrates, which are essential for biofuel production. The findings from this project indicate that bamboo can serve as a viable and renewable feedstock for the biofuel industry, contributing to sustainable energy solutions. Further optimization of the enzymatic hydrolysis and fermentation process could enhance sugar yields even further, making bamboo an attractive resource in the pursuit of cleaner, renewable energy sources.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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